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Growth inhibition by adenine in temperature-sensitive mutants

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Abstract

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Adenine sulfate (mM)	T51M1		T52M8		74A	
	25°C	35°C	25°C	35°C	25°C	35°C
0	63.5	12.9	47.0	9.1	90.3	76.6
1×10^{-5}	59.3	13.7	49.1	13.5	84.6	80.6
1×10^{-4}	62.6	4.8	45.2	9.7	88.7	84.3
1×10^{-3}	40.9	0.3	23.7	0.3	88.3	88.0
1×10^{-2}	38.4	0	7.3	0	88.4	90.3
1×10^{-1}	38.7	0	5.0	0	86.7	89.4
1	12.3	0	3.9	0	81.4	80.6

Growth effect of adenine on *ad(s)* mutants (T51M1 and T52M8) and wild-type (74A). Growth is expressed in mean dry weight (mg) of mycelia grown in 100-ml Erlenmeyer flasks containing 20 ml of medium at 25°C or 35°C for 72 hours. Approximately 1×10^4 conidia were inoculated in a flask. Mean dry weight* was based on at least three flasks each.

Growth inhibition of a few species of microorganisms by adenine has been reported (Mosteller and Goldstein 1975 J. Bacteriol. 123: 750), and adenine-sensitive (*ad(s)*) mutants have been isolated in *Salmonella typhimurium* (Dala et al. 1966 J. Bacteriol. 91: 507). During the course of isolation of temperature-sensitive mutants in *Neurospora crassa*, ten *ad(s)* mutants whose growth is inhibited by adenine (250 μM) at 35°C were obtained by the filtration-concentration technique. Growth of two of these was tested in detail as shown in the accompanying table. These mutants showed poor growth in minimal medium at 35°C as compared with that at 25°C, and growth was completely inhibited with 10 μM of adenine at 35°C. High concentrations of adenine were inhibitory in these mutants at 25°C.

Adenine, adenosine and adenosine monophosphate (100 μg/ml) were effective inhibitors of growth of *ad(s)* mutants, but other purines, pyrimidines, nucleosides and nucleotides showed no effect. Growth inhibition by adenine was not reversed by adding urines, pyrimidines, 20 essential amino acids, or vitamin stock solution including pantothenate and thiamine individually or as mixtures in the minimal medium containing adenine. Yeast extract supplemented in the minimal medium containing adenine was occasionally effective to restore growth of *ad(s)* mutants but was not readily reproducible.

A complementation test was made by inoculating ten *ad(s)* strains pairwise on minimal medium supplemented with adenine (250 μM). Two complementation groups were found, one involved mutant strains T51M1, T52M15 and T52M26, and the other T52M6, T52M8, T52M9, T52M10, T52M11, T52M12 and T52M13. The *ad(s)* mutants showed a linkage relationship with *col-4* on linkage group IV.

Adenine inhibition of adenine-sensitive mutants of *S. typhimurium* was completely relieved by either thiamine, pantothenate or methionine, and it has been suggested that adenine may inhibit the synthesis of these agents at the level of a common factor required for their syntheses. Further studies are obviously required to elucidate the nature of temperature-sensitivity and the exact site of action of adenine in *ad(s)* mutants of *Neurospora*. - - - Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.